POLYCOMBLIKE: A GENE THAT APPEARS TO BE REQUIRED FOR THE NORMAL EXPRESSION OF THE BITHORAX AND ANTENNAPEDIA GENE COMPLEXES OF *DROSOPHILA MELANOGASTER*

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ABSTRACT

A newly identified gene is described that is required for the maintenance of normal identities in many of the body segments of the fly. The effects of mutants in this gene, which is called Polycomblike (*Pcl*), suggest that its wild-type allele functions in the regulation of the bithorax gene complex (BX-C) and the Antennapedia gene complex (ANT-C). Evidence in favor of this idea derives from (1) the close correspondence between segmental transformations caused by *Pcl* mutants and those caused by dominant gain-of-function mutants in the BX-C and ANT-C, (2) the interactions observed between *Pcl* mutants and mutants in these complexes, and (3) the dependence upon BX-C and ANT-C dosage of the severity of at least one of the transformations caused by *Pcl* mutants. Arguments are presented that the control of the BX-C and ANT-C by Pcl^+ is negative in nature. The results of clonal analysis experiments indicate that, at least for the BX-C, Pcl^+ exerts this control until late in development. Since the wild-type allele of another gene, called Polycomb (*Pc*), has previously been shown to have many of the same properties as Pcl^+, it appears that the BX-C and perhaps also the ANT-C are continuously regulated during development by at least two and probably several other genes.

THE morphological specialization of most body segments of Drosophila appears to be under the direct control of a number of genes that are organized into two clusters or gene complexes. The differentiation of posterior segments, including at least those from the third thoracic through the most posterior abdominal segment, requires the activities of genes located within the bithorax complex (BX-C). This gene complex has been studied extensively by E. B. LEWIS (for reviews see LEWIS 1978, 1981a, 1981b) who has shown that the activities of the BX-C genes promote the development of posterior characteristics; all known loss-of-function mutants within the BX-C cause particular segments, or structures within them, to develop as if they were located more anteriorly in the animal. LEWIS has mapped many inactivation-type mutants by recombination and has found a striking correlation: the order of mutants within the BX-C is the same, with one exception, as the order on the animal of the body segments that these mutants affect. The study of embryos deficient for portions of the BX-C has led LEWIS to propose that early in normal development the genes of the BX-C become activated in sequence along the chromosome as one proceeds posteriorly in the animal. Evidence has been presented (LEWIS 1978) that once activated, at least two, and perhaps all, of the BX-C genes

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remain active in all more posterior segments. To explain this ordered regulation, LEWIS has proposed that each structural gene in the BX-C is controlled by adjacent *cis*-regulatory regions that are in some way able to sense location along the anteroposterior axis. These *cis*-regulatory sites are thought to be identified by dominant gain-of-function mutants in the BX-C, all of which cause posteriorly directed segmental transformations. LEWIS (1978) has presented genetic evidence that at least two of these gain-of-function mutants cause such transformations by altering the regulation of adjacent BX-C genes so that they are expressed more anteriorly than they normally are.

Recently, T. KAUFMAN and his colleagues (KAUFMAN, LEWIS and WAKIMOTO 1980) have presented evidence that the anterior portion of the fly, including the head region and the first and second thoracic segments, is under the control of another gene cluster that they have named the Antennapedia complex (ANT-C). Although the regulatory rules for this gene complex have not yet been well characterized, certain similarities to the BX-C exist. For example, *cis*-regulatory mutants appear to occur in the ANT-C (DENELL *et al.* 1981; STRUHL 1981a), and at least one of the genes in this complex appears to be regulated along the body axis in a fashion similar to that seen for some of the BX-C genes (DUNCAN, 1982).

A central problem is how the genes of the ANT-C and BX-C come to be differentially expressed along the body axis. LEWIS (1978) has proposed how such spatial control of the BX-C may occur. He has found that mutants at the Polycomb (*Pc*) locus, which is not closely linked to either the ANT-C or the BX-C, have properties suggesting that this gene is required in normal development to inactivate BX-C functions in anterior body segments. Thus, many or all of the BX-C genes appear to be expressed in many or all of the body segments of embryos deficient for Pc^+, resulting in transformations of these segments toward the most posterior, or eighth, abdominal segment. Posteriorly directed transformations are also seen in adult animals carrying only one dose of Pc^+. These include partial conversions of wing to haltere and patchy transformations of the first, fourth, fifth and sixth abdominal segments to the next most posterior segments. Since the severity of many of these transformations has been shown to depend on BX-C dosage (LEWIS 1978; DUNCAN and LEWIS 1981), it seems likely that these transformations result from the defective regulation of the BX-C and are not a direct consequence of the inactivation of Pc^+.

To explain the effects of *Pc* mutants, LEWIS (1978) has proposed that Pc^+ is a negative regulator of BX-C genes and that early in development a gradient of Pc^+ activity is set up along the body axis with its high point in the anterior of the animal. In this model, LEWIS considers the genes of the BX-C to be differentially sensitive to repression by Pc^+, with the consequence that BX-C genes with increasing sensitivities to repression by Pc^+ become sequentially activated as one proceeds posteriorly in the animal.

In addition to BX-C related transformations, adults heterozygous for *Pc* mutants often show partial conversions of antennae into legs and transformations of second and third legs into first legs. Because these transformations are characteristic of certain dominant gain-of-function mutants in the ANT-C, it seems likely that this gene complex is also controlled by Pc^+. This idea is supported by the observation that the frequencies of particular segmental transformations in *Pc*-mutant heterozygotes depend upon ANT-C dosage (DUNCAN and LEWIS 1981).

In order to better understand how the ANT-C and BX-C are controlled, a systematic search has been made for other genes that appear to act as regulators of these gene complexes. In this report, the properties of one of the genes identified in this search are described. This locus appears to be very similar in function to Pc^+ and has, therefore, been called Polycomblike (*Pcl*). Mutants in this gene cause a number of segmental transformations that correspond closely to those caused by the known dominant gain-of-function mutants in the ANT-C and BX-C. This and the observation that the severity of particular segmental transformations in *Pcl*-mutant animals depends upon ANT-C and BX-C dosage suggest that Pcl^+, like Pc^+, is required for the normal control of these gene complexes. Clonal analysis experiments indicate that, at least for the BX-C, Pcl^+ exerts this control until late in development.

MATERIALS AND METHODS

Culture conditions: Routine cultures were maintained in half-pint milk bottles or in vials on the medium of LEWIS (1960) at 25°C and approximately 80% humidity. Cultures used in the mutagenesis and clonal analysis experiments were supplemented with live baker's yeast. Unless otherwise indicated, all mutants are described in LINDSLEY and GRELL (1968).

Isolation of mutants: *Canton-S* (*C-S*) males of various ages were treated with ethyl methanesulfonate (EMS) as described by LEWIS and BACHER (1968) or were X-rayed with 4200 r using a Westinghouse 250-kv industrial unit (dose rate 400 r/min, 220 kv, 14 mA, unfiltered). These males were mated to *In(3R)85C-E;88E-F,*Pc^3*/Df(3R)Ubx*^{109} (both chromosomes described in LEWIS 1980) females and were transferred to fresh media every second day until the sixth, when they were discarded. The *In(3R)85C-E;88E-F* chromosome was used here to effectively balance Pc^3 with respect to *Df(3R)Ubx*^{109}. The number of offspring was estimated by counting all animals emerging from every tenth bottle. Although progeny from these crosses were examined to detect mutations that interact with either Pc^3 or *Df(3R)Ubx*^{109}, only those interacting with Pc^3 are described in this report. Likely mutant-bearing individuals were mated to *In(3R)85C-E;88E-F,*Pc^3*/TM1* animals and those breeding true were kept in stock balanced by *CyO* or *TM1*.

Mapping: All mutant chromosomes were examined in salivary gland preparations by standard methods. In order to detect translocations with heterochromatic breakpoints each mutant was tested for meiotic segregation in males from the markers *net* (chromosome 2), sbd^2 (chromosome 3) and spa^{pol} (chromosome 4). Second-chromosome mutants were mapped by recovering crossovers between mutant-bearing chromosomes and an *al dp b pr c px sp* chromosome. Both crossover types were recovered in each interval and were tested for their interactions with Pc^3.

Clonal analysis: Cultures destined for irradiation of larvae were maintained in half-pint milk bottles that had been cut transversely just above the level of the food. The two halves of the bottle were kept sealed together with "Time" tape except during irradiation. All cultures were treated with approximately 1000 r using a Westinghouse 250 kv unit (dose rate 400 r/min, 220 kv, 14 mA, unfiltered) and only those animals treated 24–28 hr before pupation were examined. Abdomens and wings were mounted and inspected for clones as described below.

Adult morphological examinations: Flies were preserved in a solution of one part glycerol in three parts 95% alcohol by volume. Heads and legs were dissected away from the bodies of preserved flies, cooked for approximately 5 min in 10% KOH solution at the temperature of boiling water, boiled in distilled water, dehydrated in *n*-propanol, and mounted in euparal "Vert" on a slide under a coverslip. Wings were plucked from flies, washed in distilled water, dehydrated in *n*-propanol, and mounted in euparal "Vert". Spermathecae were dissected from the abdomens of freshly killed females, boiled briefly in 10% KOH, placed under a coverslip, and photographed while still in 10% KOH solution. Abdomens were pulled from the thoraces of preserved flies with forceps, split mid-dorsally with a razor blade, and placed in a drop of 10% KOH on a microscope slide. These were arranged with forceps so that the hemitergites were spread out as in a fan. They were then squashed under coverslips and placed on a slide warmer at 51°C for 3 hr. After this, abdomens were washed off the slide in a stream of distilled water, dehydrated in *n*-propanol, and mounted in euparal "Vert". All preparations were examined with a compound microscope.

Embyros: All crosses from which embryos were to be collected were maintained for approximately 1 week on food supplemented with live baker's yeast. Parents were then transferred to

Plexiglas cages and embryos were collected on pallets containing media prepared as follows (recipe from Dr. DANIELLE THIERRY-MIEG): 1000 ml distilled water and 30 g agar were autoclaved in one flask, while 500 g fresh baker's yeast and 500 ml 3.75% acetic acid (vinegar) were autoclaved in another. These were then combined and neutral red was added to color. Just before use, methyl p-hydroxybenzoate was dissolved in 95% ethanol and added (2 g dissolved in 20 ml ethanol/liter). Eggs were collected for 12 hr and counted. Unhatched eggs were scored at 48 hr and the number showing no sign of development (presumably unfertilized eggs) was noted. The chorion and vitelline membranes of embryos to be examined were removed by manipulating the animal on the sticky surface of "Time" tape. Embryos were mounted in a drop of nine parts lactic acid to one part 95% ethanol (LEWIS 1978), left on a slide warmer at 51°C for approximately 24 hr, and examined by phase contrast microscopy.

RESULTS

Isolation of mutants and their genetic characterization: To search for genes involved in regulating the BX-C and ANT-C, mutants were selected that enhance the frequency or extent of the segmental transformations found in Pc^3/+ adults. Pc^3 heterozygotes often show partial transformations of antenna to leg, second and third leg to first leg, and wing to haltere. Posteriorly directed conversions of the first, fourth, fifth and sixth abdominal segments are also often seen. Because of these incomplete transformations, it was thought that Pc^3 heterozygotes might be very sensitive to the presence of any mutants that further reduce the extent of BX-C repression or ANT-C regulation. Accordingly, after X-ray or EMS mutagenesis of parental males, Pc^3/+ progeny were examined for the strong expression of any *Pc*-associated segmental transformation. Of the mutants found, only those that act as general enhancers of Pc^3 and that are located on the second chromosome are described in this report.

From approximately 7800 animals examined after X-ray mutagenesis, one second-chromosome mutant that enhances all *Pc*-associated transformations was recovered. Examination of about 6800 individuals after EMS mutagenesis led to the isolation of three such mutants. All mutant-bearing chromosomes are normal in salivary gland preparations and the results of segregation tests indicate that none carry translocations with heterochromatic breakpoints.

The four mutants appear to be allelic. They fail to complement one another (heterozygous combinations are either lethal or surviving adults show marked segmental transformations) and each mutant maps to approximately the same locus at map position 84 (based on a combined total of 135 recombinants between *c* and *px*). For reasons presented below, the gene identified by these mutants has been called Polycomblike (*Pcl*).

Two deletions for *Pcl* have been recovered by E. B. LEWIS in the course of experiments in which X-ray-induced mutants were selected that enhance the normally weak wing-to-haltere transformation present in flies of the genotype *CbxUbx*/++. One deletion, called *Df(2R)11B*, removes material from 54F6-55A1,2 to 55C1-3, whereas the other, called *Df(2R)7B*, extends from 54E8-F1 to 55B9-C1. These deletions act as general enhancers of Pc^3 and in complementation tests behave identically to the two strongest *Pcl* alleles. The *Pcl* locus, therefore, is located on chromosome arm *2R* between 54F6 and 55C1 on the salivary chromosome map and the *Pcl* point mutants all appear to be hypomorphic or amorphic alleles. Animals heterozygous for a wild-type second chromosome and any of the *Pcl* mutants or deficiencies often show weak transformations of their second and third legs into first legs (as evidenced by

the development of sex comb teeth on the posterior legs of some males) and partial transformations of their fourth abdominal segments into fifth segments (as shown by the development of pigmented patches on the fourth tergites of some males). *Pcl* is, therefore, a haplo-abnormal gene.

Tests of allelism of *Pcl* mutants with known homeotic mutants located in 2*R* indicate that *Pcl* is a previously undescribed gene. None of the *Pcl* mutants interacts with Hexaptera (located distal to *en* by LEWIS, unpublished) in heterozygotes. *Df(2R)11B* does not interact with Opthalmoptera-B (Opt^B) (LEWIS and BACHER 1969) or Opt^G (GARCIA-BELLIDO 1969) in the presence or absence of ey^D (which enhances the expression of Opt^B). The cytological location of *Pcl* rules out allelism with engrailed (KORNBERG 1981) or bicaudal (NUSSLEIN-VOLHARD 1977).

Properties of Polycomblike mutant alleles: The Polycomblike point mutants have been named Pcl^1, Pcl^2, Pcl^3, and Pcl^4. Pcl^1 (the only X-ray-induced mutant) and Pcl^2 behave identically. They interact strongly with Pc^3 and are lethal when homozygous or when heterozygous with one another. It is likely that these alleles are amorphic or extremely hypomorphic because they are indistinguishable from *Df(2R)11B* and *Df(2R)7B* in complementation tests. Pcl^3 and Pcl^4 are weak alleles, each surviving when homozygous or when heterozygous with Pcl^1 or Pcl^2. Viable genotypes show in the adult partial transformations of the second and third legs to first legs and posteriorly directed transformations of the abdominal tergites. In order of decreasing severity of segmental transformations these genotypes are: $Pcl^1/Pcl^3 > Pcl^1/Pcl^4 > Pcl^3/Pcl^3 > Pcl^3/Pcl^4 > Pcl^4/Pcl^4$. This sequence covers a broad range; whereas Pcl^4 homozygotes are almost wild type, the Pcl^1/Pcl^3 combination is semi-lethal with survivors showing extreme transformations of their abdominal tergites and posterior legs. Although no striking differences were seen when heterozygous combinations of *Pcl* alleles were raised at 18°, 26° and 29°, combinations including Pcl^4 show slightly more extreme leg transformations when raised at 29°, indicating that this allele is weakly temperature sensitive. Pcl^1/Pcl^4 adults show roughly the same frequency of transformation (at least for the conversion of fourth abdominal tergite to fifth) whether they are produced by $Pcl^1/+$ or Pcl^1/Pcl^4 mothers.

Description of adult segmental transformations caused by Pcl *mutants*: Pcl^1/Pcl^3 adults were chosen for detailed morphological examination. Such animals are severely affected and most die as pharate adults. Rarely, outgrowths of leg tissue (as evidenced by the presence of bracted bristles) occur on the antennae of these animals. Otherwise, all head structures appear normal. The prothorax, including the first leg and dorsal prothorax as defined by FERRIS (1950), appears normal as do the humerus and mesonotum. The fifth wing vein often fails to reach the posterior wing margin, which possibly indicates a weak transformation of wing to haltere (see clonal analysis results). The dorsal metathorax appears normal.

In most Pcl^1/Pcl^3 animals almost the entire anterior compartment of the second leg (see STEINER 1976 and LAWRENCE, STRUHL and MORATA 1979 for the locations of leg anteroposterior compartment boundaries and for good descriptions of leg morphology) is transformed into a first leg anterior compartment. The posterior compartment of the second leg, however, contains a mixture of first, second and third leg posterior structures. First leg structures include a few medium sized bristles that often occur in the central part of the posterior

compartment of the second leg femur and a row of large bristles that develops at the ventral edge of this region. The presence of trichomes but not bristles over most of the posterior compartment of the second leg femur indicates that this region is at least partly composed of normal second leg cuticle (the homologous region in the first leg femur has many more bristles and in the third leg has no trichomes). Third leg characteristics that occur in the posterior second legs of Pcl^1/Pcl^3 animals include: the frequent absence of the posterior apical coxal bristle (normally present only on first and second legs), the presence of very fine bristles in the proximal central portion of the femur posterior compartment (normally found only on third legs), and the presence of transverse rows of bristles in the posterior of the basitarsus (also normally found only on the third leg).

As was found for the second leg, the transformation of third leg to first leg in Pcl^1/Pcl^3 animals occurs primarily in the anterior compartment. This transformation is complete distally, with sex combs of 9–13 teeth forming in the basitarsus, but is incomplete proximally, with the coxa being only slightly affected. The posterior compartment of the third leg appears almost normal. There is however, some indication of a transformation of this region to first or perhaps second leg. Occasionally, a bristle forms in the central region of the trochanter posterior compartment, a feature characteristic of first and second legs. Also, small groups of trichomes often occur in the central portion of the femur posterior compartment (which is normally devoid of trichomes) and the bristles at the ventral edge of this region are slightly enlarged (a first leg characteristic). The major transformations seen in legs of Pcl^1/Pcl^3 animals are shown in Figure 1, where midventral views of basitarsae from the three legs of mutant and wild-type males are compared.

All of the abdominal tergites of Pcl^1/Pcl^3 animals show variegated transformations toward the posterior. The first abdominal tergite, which is unique in normally having only small bristles, often contains patches of cuticle that are transformed to second, third, or perhaps fourth tergite (these are indistinguishable in small patches). No fifth tergite tissue (recognizable in males by black pigmentation and presence of trichomes) has ever been seen in the first tergite. The second, third and fourth tergites often show patches of cuticle transformed to fifth or sixth (recognizable by absence of trichomes) tergites. The fifth tergite is usually largely transformed to sixth and the sixth to seventh. In males, which normally have no seventh tergite (SANTAMARIA and GARCIA-BELLIDO 1972), the latter transformation results in the loss of the sixth tergite. The transformations described for the tergites of Pcl^1/Pcl^3 animals also occur ventrally and can be seen by changes in sternite morphology. The abdominal cuticle from a typical mutant male is compared with that from a wild-type male in Figure 2.

The external genitalia and analia of mutant males and females are normal. Internally, however, all females show a transformation of their parovaria into spermathecae. Mutant females have, in addition to a pair of full-sized spermathecae, a pair that is, in general, incompletely formed. Parovaria are never present, which indicates that these extra spermathecae develop in their place and do not arise by branching of the normal spermathecal stalks, as often occurs in wild type or in females carrying the mutant *spt* (HADORN and GRABER 1944). Because surviving Pcl^1/Pcl^3 females die shortly after eclosion, it is not known if the transformation of parovaria into spermathecae has any effect on fertility.

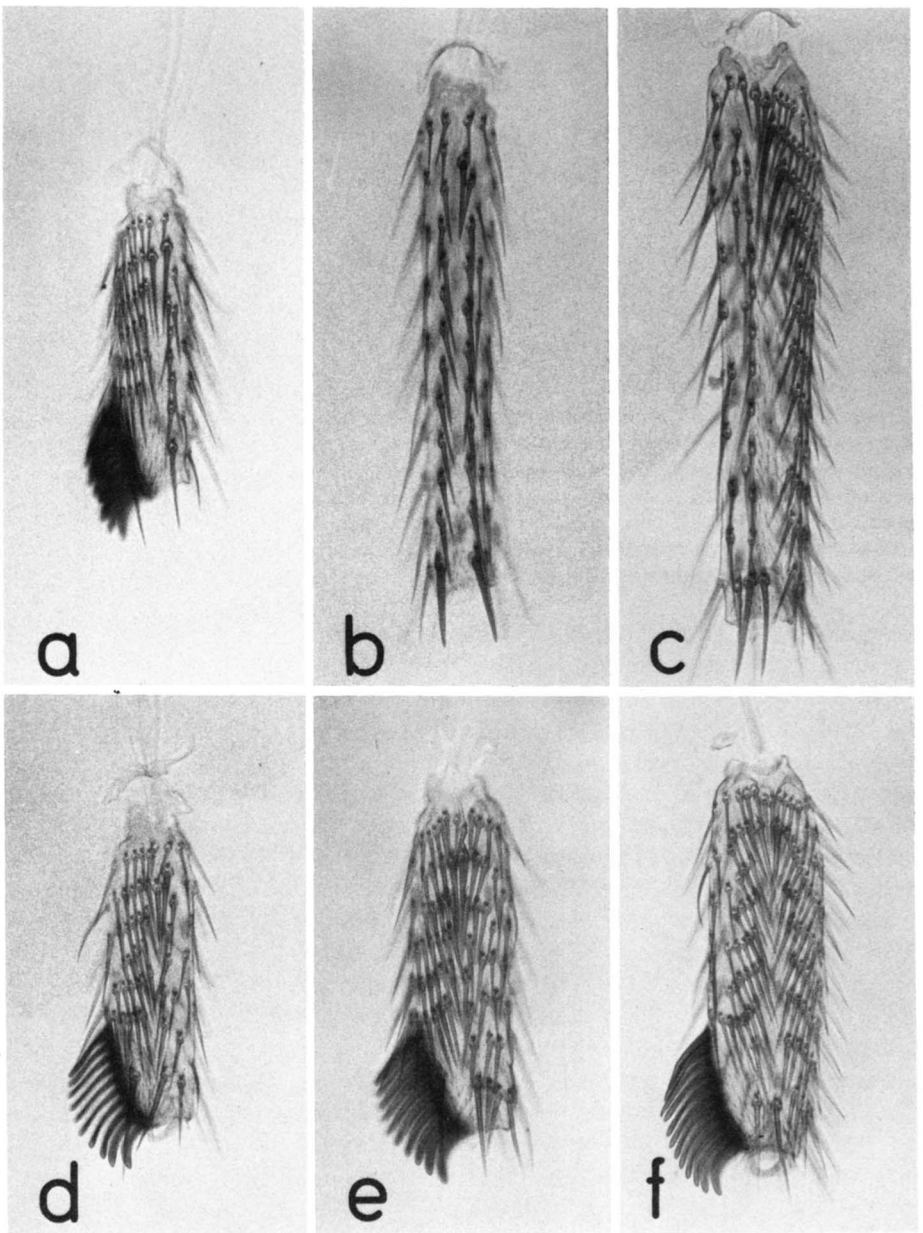

FIGURE 1.—Comparison of basitarsae from the three legs of wild-type and Pcl^1/Pcl^3 males. All are midventral views shown in the same orientation, with proximal toward the top and anterior toward the left of each figure. *a, b, c*—Basitarsae from the first, second and third legs, respectively, of wild-type males. Note that transverse bristle rows form in the anterior of the first leg basitarsus and in the posterior of the third leg basitarsus only. The anterior-posterior compartment boundary has been found by LAWRENCE, STRUHL and MORATA (1979) to be located posterior to these transverse rows in the first leg and anterior to these rows in the third leg. *d, e, f*—Basitarsae from the first, second and third legs, respectively, of Pcl^1/Pcl^3 males. Note that both anterior and posterior transverse bristle rows form in the second and third legs. These transverse bristle rows indicate that, in Pcl^1/Pcl^3 animals, the anterior portions of the second and third legs are transformed to first leg whereas the posterior portion of the second leg is transformed partially to third leg.

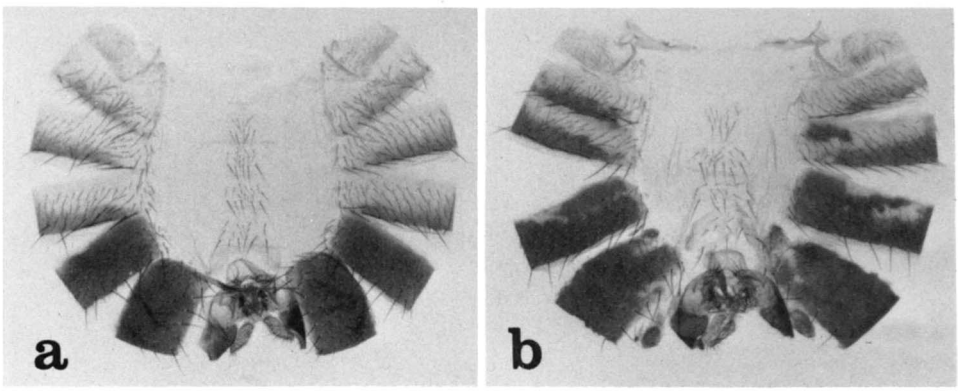

FIGURE 2.—Abdominal cuticles from a wild-type male (*a*) and a Pcl^1/Pcl^3 male (*b*). Note in (*b*) the patch of second, third or fourth abdominal segment cuticle in the first tergite and the patch of fifth or sixth segment cuticle in the third tergite. The fourth and fifth tergites are almost entirely transformed to fifth and sixth tergites, as evidenced by changes in pigmentation and trichome pattern. The sixth tergite is very reduced, presumably because this segment is largely transformed to the seventh, which in males has no tergite. The genitalia are normal. The sternites in (*b*) show posteriorly directed transformations similar to those described for the tergites.

Spermathecae from mutant and wild-type females are shown in Figure 3. The internal genitalia of males are normal.

Effects of Pcl *mutants on embryogenesis*: The data presented in Table 1 show that Pcl^1 hemizygotes or homozygotes die as embryos. These animals appear to complete embryogenesis and their cuticular structures are indistinguishable from wild type (for a description of the cuticle of wild-type embryos see LOHS-SCHARDIN *et al.* 1979). Pcl^1-homozygous embryos are morphologically wild type whether they come from mothers that are $Pcl^1/+$ or Pcl^1/Pcl^4. Animals heterozygous for *Df(2R)11B* and *Df(2R)7B* die as late embryos, but are grossly abnormal in morphology, presumably because of the absence of genes other than *Pcl*.

Pcl^1 hemizygotes that are heterozygous or homozygous for Pc^3 have also been constructed. Regardless of the parental origin of Pc^3, genotypically $Pcl^1/Df(2R)11B;Pc^3/+$ embryos are wild type in morphology. $Pcl^1/Df(2R)11B;Pc^3/Pc^3$ embryos, however, show very extreme posteriorly directed segmental transformations. Such animals are considerably more affected than are Pc^3 homozygotes that are Pcl^+. Posterior spiracle-like structures (characteristic of the eighth abdominal segment in wild type) appear in most segments. The mouth hook apparatus is almost completely undeveloped and a region of the head, probably from the labial segment, usually develops an abdominal-type ventral setal belt.

Behavior of epidermal clones homozygous for Pcl^1: Animals of the genotype *al dp pwn* $Pcl^1/+;mwh/+$ were irradiated with approximately 1000 r 24–48 hr before pupation and clones marked by *pwn* (2-58, GARCIA-BELLIDO and DAPENA 1974) or *mwh* were scored in the adult abdomen and wing. The results of this and a control experiment are presented in Table 2. In the following discussion all clones marked by *pwn* are assumed to be homozygous for the more distally located Pcl^1.

Pcl^1 homozygous clones appear to have normal viability in the abdominal tergites. In the second through the sixth abdominal segments these clones are often transformed toward the posterior. In clones homozygous for *pwn* and

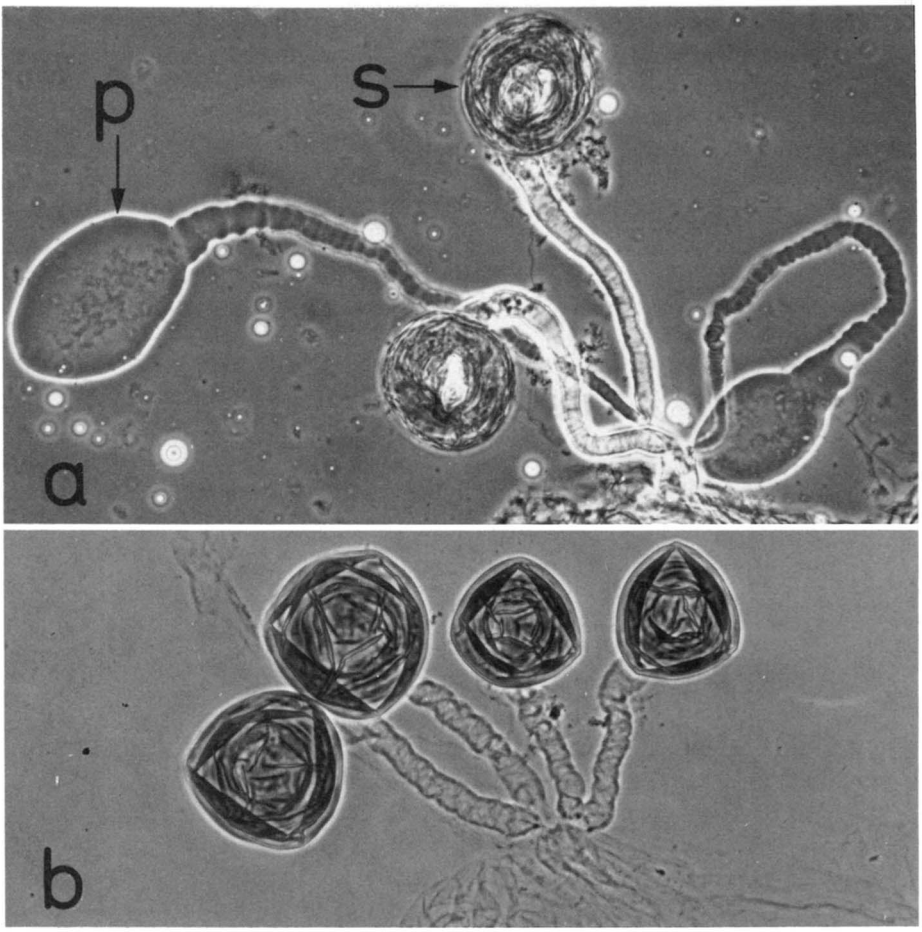

FIGURE 3.—Internal genital structures of (*a*) a wild-type (*C-S*) and (*b*) a Pcl^1/Pcl^3 female. Wild-type females possess two spermathecae (S) and two parovaria (P), whereas Pcl^1/Pcl^3 females lack parovaria and have four spermathecae. In general, one pair of spermathecae is significantly smaller than the other in Pcl^1/Pcl^3 females. The smaller spermathecae are often incompletely formed, presumably because they represent an intermediate stage in the transformation of parovaria into spermathecae.

TABLE 1

Embryonic lethality of Pcl^1 *hemizygotes and homozygotes*

Cross	Total eggs	Unfertilized eggs[a]	Embryos unhatched at 48 hr	% dead embryos
Df(2R)11B/C-S ♀♀ × Pcl^1/CyO ♂♂	288	15	77	28
Df(2R)11B/C-S ♀♀ × *C-S* ♂♂	369	17	12	3
al dp b pr c Pcl^1/*C-S* ♀♀ × Pcl^1 *px sp*/*CyO* ♂♂	288	7	67	24
al dp b pr c Pcl^1/Pcl^4 ♀♀ × *al dp b pr c* Pcl^1/CyO ♂♂	416	56	75	21

[a] Eggs showing no evidence of development at 48 hr after egg deposition are considered to be unfertilized.

TABLE 2

Behavior of Pcl^1/Pcl^1 *clones in the abdominal tergites and wing*

	Experimental genotype: *al dp pwn* Pcl^1 *px sp/+;mwh/+*				Control genotype: *al dp pwn/+;mwh/+*		
Tergite	total *pwn* clones	trans-formed *pwn* clones	*mwh* clones	N[a]	*pwn* clones	*mwh* clones	N[a]
Ab1 ♀	15	0	23	193	9	33	194
♂	6	0	16	184	13	18	183
Ab2 ♀	12	3	19	199	26	29	200
♂	11	7	16	200	12	19	198
Ab3 ♀	12	3	24	200	14	32	200
♂	17	13	21	200	16	27	199
Ab4 ♀	11	7	22	198	12	28	200
♂	9	7	19	200	18	30	200
Ab5 ♀	14	14	29	194	8	25	200
♂	9	8	18	200	17	23	200
Ab6 ♀	9	6	10	200	11	18	200
♂	0	0	2	199	6	8	200
Wing[b]	83	41	215	196	72	168	196

[a] N = number of hemitergites or wings examined.
[b] Wing clones were from *ad dp pwn* Pcl^1*/+;mwh/+* and *al dp pwn/+;mwh/+* animals.

Pcl^1, segmental transformations are almost entirely restricted to cells marked by *pwn*, indicating autonomy of Pcl^+ action. As illustrated in Figure 4, more than one segmental transformation can often be recognized within each clone.

Included in Table 2 are the proportions of Pcl^1 clones in each tergite that are detectably transformed to the posterior. Because the second, third and fourth tergites appear identical (at least over most of their surfaces), clones in these segments must be transformed to the fifth or sixth segments in males (recognizable by black pigmentation) or the sixth or seventh segments in females (recognizable by absence of trichomes) to be detectably transformed. This difference between the sexes probably explains why Pcl^1 clones in the second and third abdominal segments of females show a lower frequency of transformation than do clones in these segments of males. (Because the fifth tergite has a lower trichome density than more anterior tergites, one might expect that transformations to this segment could be seen in females. In practice, however, it is difficult to score trichome density changes in small patches.) In both sexes, clones occurring in the second and third abdominal tergites show a lower frequency of transformation than do clones in the fourth and fifth tergites, probably because short range transformations can be seen only in more posterior segments. Transformations of the sixth segment to seventh were detected in females by the loss of trichomes in Pcl^1 clones occurring in the anterior portion of the sixth tergite. This transformation probably also occurs in males (which have no seventh tergite), resulting in the absence of Pcl^1 clones in the male sixth tergite. Pcl^1 clones in the abdominal sternites show the same types of posteriorly directed transformations as do clones in the tergites.

None of the 21 Pcl^1 clones seen in the first abdominal tergite showed segmental transformations. This result is remarkable because any posteriorly directed transformation would be easily scored in the first abdominal segment. For comparison, in the fifth abdominal segment, which is also easily distin-

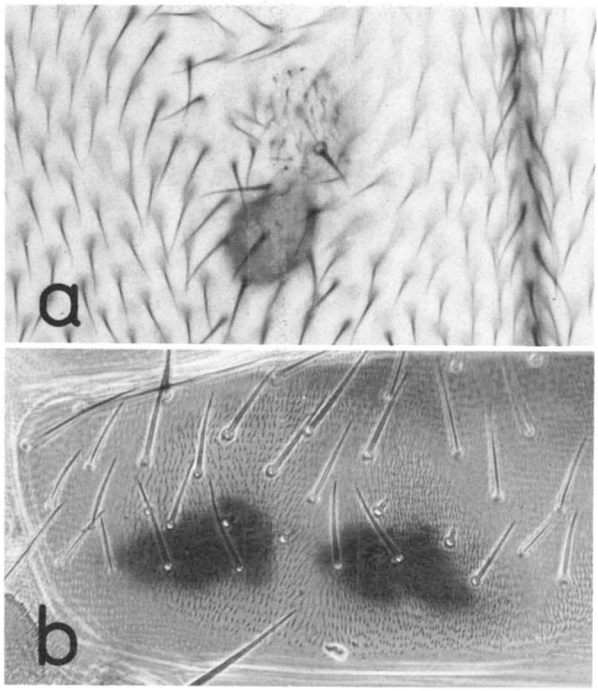

FIGURE 4.—*a*, A patch of haltere-like cuticle in the wing of an *al dp pwn Pcl*^{1}/+ female irradiated 24–48 hr before pupation. Note the presence of a bristle and small trichomes similar to those that normally occur in the haltere. A vesicle of haltere-type cuticle is also present between the dorsal and ventral surfaces of the wing blade. This sector is probably a *Pcl*^{1}-homozygous clone which arose by mitotic recombination between the loci of *pwn* and *Pcl*. In contrast, most patches of haltere-type cuticle in such wings are clearly marked by *pwn*, having arisen by mitotic recombination proximal to *pwn*. *b*, A third abdominal hemitergite from an *al dp pwn Pcl*^{1} *px sp*/+ male irradiated 24–48 hr before pupation. Shown is a clone homozygous for *Pcl*^{1} and for the cell marker *pwn*, which causes the truncation of bristles and trichomes. The clone is darkly pigmented because it is transformed to a mixture of fifth and sixth tergite. Around most of the border of the clone, pigmentation extends for a short distance into areas that have normal length trichomes, suggesting that *pwn*$^{+}$, *Pcl*$^{+}$, or the process of pigmentation itself is nonautonomous over short distances. As is often seen in tergites, cells within the clone are not contiguous. In particular, note the migration of *pwn/pwn* bristles out of the clone and the migration of wild-type bristles into the clone.

guished from all more posterior segments, 23/24 *Pcl*^{1} clones showed transformations. The wild-type appearance of clones in the first abdominal segment may indicate that the distinction of this segment from the rest of the abdomen requires activity of *Pcl*$^{+}$ only early in development. A more likely explanation, however, is that this gene never plays a major role in this segment. This idea is supported by the observation that in *Pcl*^{1}/*Pcl*^{3} animals the first abdominal segment is transformed to the posterior much less frequently than are the other abdominal segments. In such animals only about one-third of first abdominal hemitergites (13/37 examined) show any transformed cuticle (recognizable by the presence of large bristles) whereas, for comparison, the fourth abdominal tergites are always almost entirely transformed to the posterior. It seems likely, therefore, that if more *Pcl*^{1} clones had been examined in the first abdominal segment, some of them would have shown posteriorly directed transformations.

Pcl^{1} clones in the wing have normal viability and often appear to be partially

transformed to haltere, as indicated by the development of small, densely packed trichomes similar to those that occur on the haltere and, occasionally, of small bristles like those found on the haltere. An example of such a clone is shown in Figure 4. The morphology of these clones is the same in the anterior and posterior compartments of the wing.

Evidence for the interaction of Pcl^+ *with the ANT-C and BX-C*: That the segmental transformations present in Pcl^1/Pcl^3 animals resemble closely those caused by certain dominant gain-of-function mutants in the ANT-C and BX-C (see DISCUSSION) suggests that Pcl^+ interacts with both of these gene complexes. Further evidence supporting this notion has come from experiments which indicate that the severity of segmental transformations in *Pcl* mutant animals depends upon the dosage of the ANT-C and BX-C. Pcl^1/Pcl^4 males were chosen for these tests because these animals have good viability and show partial transformations of their fourth abdominal tergites to fifth tergites and of their second and third legs to first legs. Rearrangements used in the BX-C dosage tests were *Df(3R)P9(=Df(3R)89E1;89E4-5)*, which is deficient for the entire BX-C (LEWIS 1978), and *Dp(3;3)P5(=Dp(3;3)89E1,2;90A)*, which is a direct tandem duplication for the BX-C and surrounding material (LEWIS unpublished). ANT-C dosage was varied using *Df(3R)Scr(=Df(3R)84A1;84B1)*, a deficiency for most or all of the ANT-C (R. A. LEWIS *et al.* 1980) and *Dp(3;3)D1(=Dp(3;3)84A;85A)*, a direct tandem duplication that includes at least the *Scr* and *Antp* loci of the ANT-C (DUNCAN unpublished). To reduce the effects of differences in genetic background, male parents used in the dosage tests were from stocks maintained as Pcl^4*pxsp/SM1;Dp(3;3)P5,Sb/Df(3R)P9* or as Pcl^4*pxsp/SM1;Dp(3;3)D1/Df(3R)Scr,* p^p.

The results of the dosage tests are presented in Table 3. Within the range of doses tested, the data indicate that the frequency of transformation of fourth abdominal tergite to fifth in Pcl^1/Pcl^4 animals is enhanced by increasing ANT-C dosage but is markedly suppressed by increasing BX-C dosage. DUNCAN and

TABLE 3

Dependence of segmental transformations in Pcl^1/Pcl^4 *males on BX-C and ANT-C dosage*

No. of doses	3rd chromosomes	Average no. hemitergites/ fly with Ab4 → Ab5 sector	Average rank[a] leg 2 → leg 1	Average rank[a] leg 3 → leg 1	No. males examined
BX-C					
1[b]	*Df(3R)P9/+*	1.56	2.60	1.63	48
2[c]	*C-S/+*	1.27	3.07	1.44	41
3[b]	*Dp(3;3)P5,Sb/+*	0.34	3.88	1.98	56
ANT-C					
1[d]	*Df(3R)Scr,*p^p*/+*	0.82	1.48	0.71	73
2[c]	*C-S/+*	1.27	3.07	1.44	41
3[d]	*Dp(3;3)D1/+*	1.78	3.30	2.22	67

[a] The number of sex comb teeth occurring on the second and third legs were counted and each male ranked according to the following scale: 0 = 0 teeth, 1 = 1–4 teeth, 2 = 5–8 teeth, 3 = 9–13 teeth, 4 = more than 13 teeth or the presence of more teeth on one leg than could be counted reliably under the dissecting microscope (>7).

[b] From cross of *al dp b pr c* Pcl^1/CyO ♀♀ × Pcl^4 *pxsp/SM1;Dp(3;3)P5,Sb/Df(3R)P9* ♂♂.

[c] From cross of *al dp b pr c* Pcl^1/CyO ♀♀ × Pcl^4 *pxsp/SM1;TM1/C-S* ♂♂.

[d] From cross of *al dp b pr c* Pcl^1/CyO ♀♀ × Pcl^4 *pxsp/SM1;Dp(3;3)D1/Df(3R)Scr,* p^p ♂♂.

LEWIS (1981) have reported a similar effect of varying ANT-C and BX-C dosage in $Pc^3/+$ animals. These authors have also found that increasing ANT-C dosage enhances the frequency of transformation of the first abdominal segment to a more posterior (second, third or fourth) abdominal segment in Pc^3 heterozygotes. Indeed, otherwise wild-type animals carrying three or four doses of the ANT-C occasionally show posteriorly directed transformations of the first abdominal segment. The latter two effects of ANT-C duplications have been shown to be suppressed by *Df(3R)Scr*, indicating that the dosage sensitive gene(s) responsible is in or near the ANT-C. Since only specific transformations are promoted by extra doses of the ANT-C, it seems unlikely that these transformations result from the competition of the ANT-C and BX-C for a negative regulator common to all genes in both complexes. Rather, DUNCAN and LEWIS (1981) have suggested that these transformations result from the action of a gene within the ANT-C that is a positive regulator of certain BX-C genes. This idea has received support from the discovery of mutants in the ANT-C (which will be described elsewhere) that cause certain BX-C related segmental transformations.

The dependence upon BX-C and ANT-C dosage of the transformation of the posterior legs to first leg (as measured by sex comb teeth counts) in Pcl^1/Pcl^4 males is similar to that described for Pc^3 heterozygotes by DUNCAN and LEWIS (1981). Increasing BX-C dosage appears to weakly enhance this transformation (at least in the second leg) while increasing ANT-C dosage has little effect. Transformations to first leg are weak in Pcl^1/Pcl^4 males carrying a single dose of the ANT-C, but this effect is to be expected because males with only one dose of the ANT-C also show a marked reduction in the number of sex comb teeth on their first legs (KAUFMAN, LEWIS and WAKIMOTO 1980).

Further evidence for the interaction of Pcl^+ with the ANT-C and BX-C is supplied by the observation that *Pcl* mutants enhance certain dominant gain-of-function mutants in both complexes. Females heterozygous for Pcl^1 were mated to males carrying the BX-C mutants *Cbx*, Uab^1, Uab^2, Uab^4, *Hab* and *Mcp* (all described in LEWIS 1978), and the ANT-C mutants $Antp^{73}$ (GREEN 1975), *Scx*, and *Msc*. Progeny from these crosses showed that Pcl^1 causes unequivocal enhancement of *Cbx*, *Mcp*, *Scx*, and *Msc*. A particularly striking interaction is the enhancement of *Cbx*: $Pcl^1/+$; *Cbx*/+ animals show an almost complete wing-to-haltere transformation, whereas *Cbx*/+ flies usually show this transformation in the posterior of the wing only. Pcl^1 has also been shown to partially suppress the haltere-to-wing transformation present in animals homozygous for the recessive loss-of-function BX-C mutant *bxd*.

The relation of Pcl^+ *to* Pc^+: In Table 4 representative data are shown illustrating that Pcl^1 and Pc^3 markedly enhance one another. This interaction does not depend upon the *Pcl* or *Pc* alleles used nor does it depend upon their parental origin (data not shown). For each of the segmental transformations scored, the interaction appears to be synergistic rather than additive, suggesting that Pcl^+ and Pc^+ are related in function. That Pc^+ and Pcl^+ have qualitatively different functions, however, is indicated by the results presented in Table 5. In these experiments, extra doses of Pc^+ were introduced using *Dp(3;3)C126*, a direct tandem duplication for the material from approximately 78D to 79B which was isolated and shown to contain two doses of Pc^+ by M. CROSBY (unpublished). As can be seen in Table 5, this duplication has no striking effect on the phenotype of Pcl^1/Pcl^4 animals, although as one proceeds from two to

TABLE 4

The interaction between Pcl^1 *and* Pc^3

Genotype	N[a]	Avg. rank[b] ant → leg	Avg. rank[c] wing → halt.	Avg. rank[d] leg 2 → leg 1	Avg. rank[d] leg 3 → leg 1	Avg. no hemitergites/fly Ab4 → Ab5
$Pcl^1/+$[e]						
♂	28	0	0	0.18	0.04	0.07
♀	28	0	0			
$Pc^3/+$[f]						
♂	40	0	0.50	0.95	0.55	0.63
♀	41	0.5	0.80			
$Pcl^1/+;Pc^3/+$[g]						
♂	32	0.34	1.72	3.75	2.41	1.97
♀	39	1.08	2.05			

[a] N = number of animals scored.

[b] Each animal was assigned a rank based on its more extremely affected antenna according to the following scale: 0 = wild type, 1 = tiny outgrowth, 2 = moderate outgrowth, and 3 = large outgrowth.

[c] Each animal assigned rank based on more extremely affected wing according to the following scale: 0 = wild type, 1 = posterior wing slightly serrated, 2 = posterior wing serrated along most of posterior edge, 3 = posterior wing crumpled with wing abnormally curved, and 4 = haltere cuticle obvious at posterior of wing.

[d] As in Table 3.

[e] From cross of *C-S* ♀♀ × Pcl^1/CyO ♂♂.

[f] From cross of Pc^3/TMI ♀♀ × *C-S* ♂♂.

[g] From cross of Pc^3/TMI ♀♀ × Pcl^1/CyO ♂♂.

TABLE 5

Dependence of segmental transformations in Pcl^1/Pcl^4 *males on* Pc^+ *dosage*

No. of Pc^+ doses	3rd chromosomes	Avg. no. hemitergites/fly with Ab4 → Ab5 sector	Avg. rank[a] leg 2 → leg 1	Avg. rank[a] leg 3 → leg 1	No. males examined
2[a]	*C-S/+*	1.27	3.07	1.44	41
3[b]	*Dp(3;3)C126, in ri* $p^p/+$	0.84	2.59	1.11	56
4[b]	*Dp(3;3)C126, in ri* $p^p/$ *Dp(3;3)C126, in ri* p^p	0.74	2.36	0.83	47

[a] As in Table 3.

[b] From cross of *al dp b pr c* $Pcl^1/+$;*Dp(3;3)C126, in ri* $p^p/+$ ♀♀ × Pcl^4 *px sp/*Pcl^4 *px sp;Dp(3;3)C126, in ri* $p^p/TM3$, *Sb Ser* ♂♂.

four doses of Pc^+ there appears to be a trend towards suppression of segmental transformations in these animals. *Dp(3;3)C126* is a moderately large duplication, however, and this suppression may result from genes other than Pc^+ that are contained within it. In any case, Pc^+ activity does not appear to be able to substitute for Pcl^+ activity, at least within the range of doses tested.

DISCUSSION

A gene that appears to be required for the maintenance of normal segmental identities in most (perhaps all) body segments is described in this report. This gene has many of the same properties as Polycomb (*Pc*) and has therefore been called Polycomblike (*Pcl*). The analysis of *Pcl* mutants suggests that the wild-

type allele of this locus is involved in the control of the ANT-C and BX-C, two gene clusters thought to be directly responsible for the specialization of most body segments.

The main line of evidence for the involvement of Pcl^+ in the control of the ANT-C and BX-C is that the segmental transformations caused by *Pcl* mutants closely correspond to those caused by dominant gain-of-function mutants in these complexes. For example, the transformation of antenna into leg, which is occasionally seen in Pcl^1/Pcl^3 animals, is also caused by a number of dominant mutants in the ANT-C which, for the most part, have been considered Antennapedia (*Antp*) alleles. Transformations of second and third legs into first legs are caused by two other dominant ANT-C mutants called Extra sex comb (*Scx*) and Multiple sex comb (*Msc*). Although each of these mutants is lethal when homozygous, *Scx/Msc* heterozygotes survive to adulthood and show much stronger leg transformations than those seen in animals carrying either mutant alone. Microscopic examination of the posterior legs from *Scx/Msc* animals has revealed a pattern of conversion to first leg that is almost identical to that found in Pcl^1/Pcl^3 animals [with the exception that, because of an *Scr* mutation associated with *Msc* (R. A. LEWIS *et al.* 1980), the number of sex comb teeth and bristles of the anterior tarsal transverse rows is reduced in all legs of *Scx/Msc* as compared to Pcl^1/Pcl^3 animals]. In the second legs of *Scx/Msc* animals the anterior compartment is almost completely transformed to first leg, whereas the posterior compartment is only partially so, as evidenced by the development of an incomplete set of first leg bristles in the femur posterior compartment. The anterior compartment of the third legs of *Scx/Msc* animals is completely transformed to first leg distally, but is almost normal proximally. The third leg posterior compartment appears to be unaffected. The preferential transformation of the anterior compartments of the second and third legs to first leg is also seen in males heterozygous for *Pc* mutants and, at least for the third leg, apparently also in males homozygous for $l(3)1902^{OX736hs}$ after exposure to the restrictive temperature in the third larval instar (SHEARN, HERSPERGER and HERSPERGER 1978). Unlike Pcl^1/Pcl^3 animals, *Scx/Msc* adults do not show any transformation of second leg to third leg.

The remaining transformations described for *Pcl* mutant animals correspond to those caused by dominant gain-of-function mutants located in the BX-C. Transformation of the second thoracic segment to third thoracic segment, as seen in the wing (in Pcl^1/Pcl^1 clones) and in the posterior compartment of the leg (in Pcl^1/Pcl^3 adults), is caused by the BX-C mutant Cbx^2 (LEWIS 1981b). (Cbx^1, although causing a partial transformation of anterior and posterior wing to haltere, does not affect the posterior compartment of the second leg.) Posteriorly directed transformations of the abdominal segments are caused by mutants in the *Uab* group of the BX-C (which transform the first abdominal segment to a more posterior segment) and by *Mcp* (which converts the fourth abdominal segment into the fifth). Finally, the transformation of parovaria into spermathecae, as described for Pcl^1/Pcl^3 females, is often seen in Pc^3/Uab^1 females (approximately 50% of such females show at least one parovarium transformed to a spermatheca). This transformation has not been seen in either $Pc^3/+$ or $Uab^1/+$ females (over 30 of each type examined). Although cytologically normal, Uab^1 suppresses recombination over most of the BX-C (LEWIS unpublished), which suggests that this mutant is a rearrangement involving at

least two breakpoints within the BX-C. Because heterozygotes for Pc^3 and other mutants of the *Uab* type do not show transformation of parovaria to spermathecae, it seems likely that this transformation is promoted by a different breakpoint of Uab^1 than is the transformation of the first abdominal segment to the second. Moreover, because $Pc^3/Df(3R)P9$ (*Df(3R)P9* is deficient for the entire BX-C) females do not show transformation of parovaria to spermathecae (over 30 females examined), it is likely that Uab^1 promotes this transformation by a gain rather than a loss of function in the BX-C. This conclusion is supported by the finding of the reverse transformation, the conversion of spermathecae into parovaria, in females heterozygous for $In(3LR)Uab^4$ and *T(2;3)P10* (these rearrangements, which both have breakpoints within the BX-C, are described in LEWIS 1978). $In(3LR)Uab^4/T(2;3)P10$ animals have been described by LEWIS (1978) as showing transformations of the third, fourth and fifth abdominal segments to second abdominal segments. Based on these transformations, LEWIS has argued that $In(3LR)Uab^4$ and *T(2;3)P10* inactivate at least partially a BX-C gene or genes required for the normal development of posterior abdominal segments. It seems likely that the inactivation of these genes is also responsible for the observed transformation of spermathecae into parovaria.

The conversion of parovaria to spermathecae is unique among transformations caused by BX-C gain-of-function mutants in that it is anteriorly directed; evidence from fate mapping in Drosophila (SCHUPBACH, WIESCHAUS and NOTHIGER 1978) and from embryological examination of other dipterans (DUBENDORFER 1970, 1971; EMMERT 1972) indicates that spermathecae lie anterior to parovaria at the time of their formation. Based on extensive studies of the internal reproductive structures of species in Drosophila and related genera, Dr. L. THROCKMORTON (personal communication; see also THROCKMORTON 1962) has formed the opinion that spermathecae have evolved from parovaria-like structures. The transformations of parovaria to spermathecae described above may be consistent, therefore, with the generalization that the activities of BX-C genes promote the development of evolutionarily more advanced characteristics.

The nearly complete identity between the transformations caused by *Pcl* mutants and those caused by ANT-C and BX-C dominant gain-of-function mutants suggests that some interaction takes place between Pcl^+ and these gene complexes. The idea that Pcl^+ interacts with the ANT-C and BX-C is supported by the dependence of the phenotype of Pcl^1/Pcl^4 animals on ANT-C and BX-C dosage and by the enhancement of certain gain-of-function mutants in both complexes by Pcl^1. Because *Pcl* mutants are pleiotropic, whereas ANT-C or BX-C mutants cause only particular transformations, it seems more reasonable to propose that Pcl^+ in some way regulates genes in these complexes rather than the reverse. The nature of this regulation by Pcl^+ is clear only for the BX-C. In this complex, all known loss-of-function mutants cause particular segments or parts thereof to develop as more anterior ones. Therefore, BX-C related transformations of segments toward the posterior, such as those that occur in Pcl^1/Pcl^3 animals, can be reasonably interpreted as resulting from hyperactivity of BX-C genes. Since the *Pcl* mutants studied appear to be hypomorphic or amorphic alleles, one of the normal functions of this locus can be thought of as repression of BX-C genes. A similar argument has been made for the function of Pc^+ by LEWIS (1978).

The nature of the control of the ANT-C by Pc^+ and Pcl^+ is not as clear as that of the BX-C. Although cases for both positive and negative control of the ANT-C by these genes can be made, in at least two instances the available evidence argues in favor of negative control. DENELL *et al.* (1981) and STRUHL (1981a) have presented evidence that the ANT-C mutant *Ns* (and perhaps all dominant mutants of the *Antp* type) causes the transformation of antenna into leg by turning on an ANT-C gene in the antenna, where in normal development it would be inactive. That animals mutant for *Pc* or *Pcl* also show this transformation suggests that Pcl^+ and Pc^+ are required to repress the activity of this gene in the antenna.

The transformations of second and third leg to first leg seen in *Pcl*- and *Pc*-mutant animals can also be interpreted as resulting from hyperactivation of an ANT-C gene. Examination of Figure 1 reveals that the first tarsal segments (basitarsae) of the three legs of wild type show a striking symmetry. Several transverse rows of bristles (one of which is modified in males to form the sex comb) are present in the anterior of the first leg basitarsus whereas a similar set of transverse rows is located in the posterior of the third leg basitarsus. No transverse rows are present on the first tarsal segment of the second leg. R. A. LEWIS *et al.* (1980) have found that animals heterozygous for mutations that inactivate the *Scr* gene of the ANT-C show a marked reduction in the number of bristles per anterior transverse row in the first leg basitarsus. The author has quantitated this effect by counting the number of bristles present in the penultimate distal transverse row in the first leg basitarsae of males carrying one, two and three doses of the ANT-C. In this bristle row, *Df(3R)Scr/+* males show an average of 4.6 bristles, wild-type (*C-S*) males show 7.5 bristles, and *Dp(3;3)Dl/+* males show 9.4 bristles (20 basitarsae of each genotype were examined). Similarly, it has been determined that the number of bristles per posterior transverse row in the third leg basitarsus is dependent upon BX-C dosage. The average number of bristles in the penultimate distal transverse row in the third leg basitarsae of *Df(3R)P9/+* males is 4.5; for wild-type (*C-S*) males it is 6.1, and for *Dp(3;3)P5/+* males it is 7.4 (based on counts of 20, 22 and 12 basitarsae, respectively). It appears, therefore, that the activity of an ANT-C gene (Scr^+) promotes the formation of anterior tarsal transverse rows whereas the activity of a BX-C gene or genes promotes the formation of posterior tarsal transverse rows. In normal development, these genes appear to be inactive in the second leg, which has no transverse bristle rows. In Pcl^1/Pcl^3 animals, however, transverse bristle rows develop in both the anterior and posterior of the second leg basitarsus, suggesting that Pcl^+ functions in this leg to repress the activities of Scr^+ and of one or more BX-C genes. In *Pc*-mutant animals, transverse bristle rows have been found in the anterior, but not the posterior, of the second leg basitarsus. Pc^+, therefore, can also be thought of as a repressor of Scr^+ activity in the second leg. Although the model that Pcl^+ and Pc^+ are negative regulators of Scr^+ is attractive, the observations of WAKIMOTO and KAUFMAN (1981) suggest that the transformations of second and third legs to first legs seen in *Pc*- and *Pcl*-mutant animals may result from a defect in the positive regulation of $Antp^+$, another of the ANT-C genes. In any case, it seems likely that the compartmental preferences shown by transformations in the legs of *Pc*- and *Pcl*-mutant animals reflect the differential activities of ANT-C and

BX-C genes in the anterior and posterior portions of the thoracic segments. Evidence for such differential expression of $Antp^+$ (DUNCAN 1982) and of certain BX-C genes (MORATA and KERRIDGE 1981; LEWIS 1978) has already been described.

Although the similar phenotypes and strong mutual enhancement of *Pcl* and *Pc* mutants suggest that these genes have very similar functions, it appears that these functions are not identical. One line of evidence suggesting this is that *Pc* and *Pcl* mutants exert their major effects on different body structures. For example, animals heterozygous for *Pc* mutants have more strongly affected wings and antennae and much more weakly transformed legs and abdomens than do Pcl^1/Pcl^3 animals. The results of clonal analysis experiments also indicate that these genes have different functions. When induced late in development, abdominal clones of cells homozygous for Pcl^1 show variable short-range transformations toward the posterior. Similar clones lacking Pc^+ activity uniformly transform to what appears to be anal plate (STRUHL 1981b; DUNCAN and LEWIS 1981), thought to be the most posteriorly derived structure in the external cuticle of the adult fly (SCHUPBACH, WIESCHAUS and NOTHIGER 1978). Since Pcl^1 appears to be a null allele, these observations indicate that Pcl^+ activity is not absolutely required for the maintenance of BX-C gene repression, whereas Pc^+ activity probably is so required. Because the clones examined in these experiments were large, it seems unlikely that the differences in behavior of clones lacking Pcl^+ and those lacking Pc^+ are due to persistence of Pcl^+ product. That extra doses of Pc^+ have no major effect on the phenotype of Pcl^1/Pcl^4 animals supports the idea that Pc^+ and Pcl^+ perform different functions.

The discovery of *Pcl* raises the question of whether other genes exist that are similar in function to Pc^+. In fact, three other such genes have been identified by selecting mutants that act as dominant enhancers of Pc^3 and by selecting mutants that modify the expression of certain BX-C mutants. These genes will be described in future reports. In addition, two genes have been described in the literature that appear similar in function to Pc^+. One of these is a gene called extra sex combs (*esc*). STRUHL (1981b) has recently reported that this gene exerts a maternal effect such that homozygous mutant progeny from homozygous mutant mothers die as mature embryos that show transformations of most of their body segments toward the most posterior abdominal segment. Animals homozygous for *Pc* mutants show similar transformations (LEWIS 1978; DUNCAN and LEWIS 1981). Unlike Pc^+, however, esc^+ does not appear to have any major function late in development [although see TOKUNAGA and STERN (1965) for a late activity of esc^+ in maintaining identities in the second and third legs]. Another gene with a function similar to that of Pc^+ is $1(4)29^+$. GEHRING (1970a, b) has reported that animals homozygous for amorphic alleles of *1(4)29* die as pharate adults that show transformations of the proximal antennal segments into proximal leg segments and partial transformations of the second and third legs into first legs. Examination of animals homozygous or hemizygous for previously described amorphic alleles or for a new spontaneous hypomorphic allele ($1(4)29^c$) has shown that these mutants also cause posteriorly directed transformations of the abdominal segments and, in females, transformation of parovaria into spermathecae. That this gene has a function somewhat different from that of either Pc^+ or Pcl^+ is suggested by the observation that *1(4)29* mutants cause transformations of only proximal antennal segments into leg and

suppress the development of the dorsal prothorax (as defined by FERRIS 1950), effects that have not been seen in animals mutant for *Pc* or *Pcl*. Although it is not clear why the fly should require several genes that are similar to Pc^+ in function, the existence of these genes should not be surprising in view of the number of genes known to be involved in other developmental processes (for example sex determination; see BAKER and RIDGE 1980).

In addition to negative control by a number of genes, the functions of the BX-C appear to be under the positive control of at least one gene. This is Regulator of bithorax (*Rg-bx*) (see GARCIA-BELLIDO and CAPDEVILA 1978), a gene discovered and localized to 88B on the polytene chromosome map by E. B. LEWIS (1968). Animals mutant for this gene show a number of segmental transformations that correspond to those caused by loss-of-function mutants in the BX-C. Patchy transformations of the haltere to wing and of the posterior abdominal segments to more anterior ones are often seen in animals heterozygous for amorphic alleles of *Rg-bx*. Homozygotes for such alleles die as late embryos or first instar larvae that show anteriorly directed transformations of their posterior abdominal segments (DUNCAN and LEWIS 1981). INGHAM and WHITTLE (1980) have described a hypomorphic *Rg-bx* allele called trithorax (*trx*) that when homozygous or hemizygous causes segmental transformations that are strikingly similar, but opposite in direction, to those seen in Pcl^1/Pcl^3 adults. For example, *trx* homozygotes show anteriorly directed conversions of the abdominal segments and partial transformations of the first and third legs to second legs. Females homozygous for *trx* also show extra spermathecae (INGHAM and WHITTLE 1980), although it is not clear if these arise by transformation of parovaria, as they do in Pcl^1/Pcl^3 females. The opposite orientations of most of the transformations caused by *Rg-bx* and *Pcl* mutants suggest that these genes control in opposite ways many of the same genes in the ANT-C and BX-C.

The picture that is emerging from recent work on genes controlling the specialization of body segments in Drosophila is that segmental identities are determined directly by genes that are clustered within the ANT-C and BX-C. Many genes that affect segmental character are located outside of these clusters, but several, if not all, of these genes can be thought of as regulators of genes within the two complexes. These regulators appear to be of at least two types: those that are active only early in development and those that act continuously. At present, our working hypothesis is that early in embryogenesis one or more of the continuously acting regulators comes to be expressed differentially along the body axis, a process which may be controlled by the early acting regulators. The activities of the continuously acting gene(s) could be regulated, for example, so that they grade from anterior to posterior in the animal, as suggested for Pc^+ by LEWIS (1978). Once established, it is proposed that the pattern of differential expression of the continuously acting regulators is maintained through later development. The existence of an anteroposterior gradient of a BX-C regulator and the maintenance of this gradient until late in development is supported by experimental evidence presented by DUNCAN and LEWIS (1981). The genes of the ANT-C and BX-C are thought to have different sensitivities to the continuously acting regulators and to respond passively at all times to them. As a result, each segment comes to be characterized by a unique subset of ANT-C and BX-C gene activities which then determines the type of specialization undergone by that segment. The role of the genes of the ANT-C and BX-C in this model is

to act as intermediaries between a few controlling genes like Pc^+ and Pcl^+ and the large batteries of genes that presumably must be regulated to elaborate structures characteristic of each body segment.

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